REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Initially, applicants would like to note that the present amendment is being submitted in compliance with "Amendments In A Revised Format Now Permitted", 1267 OG 4 (February 25, 2003). Pursuant to this notice, the requirements of 37 C.F.R. § 1.121 have been waived.

Support for the amendments to the claims is found, for example, at page 8, lines 2-9 of the specification and in the original claims.

The objection to claims 2, 3, and 11 for various informalities is obviated in view of the above amendments.

The rejection of claims 2-25 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed in view of the above amendments and the following remarks.

It is the position of the U.S. Patent and Trademark Office ("PTO") that the specification is enabling for making a mouse model showing a phenotype of pemphigus vulgaris ("PV") by transplanting activated immune cells into an immunodeficient mouse, where the activated cells are obtained by immunizing a Dsg3-/- mouse lacking a Dsg3 antigen with the Dsg3 antigen, but the specification does not provide enablement for: (1) making any non-human mammal showing a phenotype of an autoimmune disease; (2) making a non-human PV model/any non-human autoimmune model by transplanting non-activated immune cells; (3) making an autoimmune model by transplanting activated immune cells into an immune competent non-human mammal; or (4) making an autoimmune model by transplanting activated immune cells into a xenogenic immunodeficient non-human mammal. Applicants respectfully disagree.

With regard to item (1), the PTO states that practicing the invention requires making a line of transgenic non-human mammals, particularly antigen knockout non-human mammals, and that the phenotypes resulting from targeted disruption of an antigenic gene in different strains are expected to be varied and unpredictable (office action page 7, 1st full para.). Thus, the PTO argues that the skilled artisan could not practice the invention without first carrying out undue experimentation to make a homozygous knockout for a particular gene. Applicants respectfully disagree.

In contrast to the PTO's position, it is not necessary to know detailed knockout phenotypes of an antigenic gene to make or use the claimed invention. In order to practice the present invention, all that is required is to obtain immune cells from a chosen characterized knockout animal. As noted by the PTO at page 7, lines 5-6 of the outstanding office action, techniques of making knockout animals have become routine in the relevant art. Therefore, it is well within the ability of one skilled in the art to obtain immune cells from any knockout animal as long as the animal is viable.

A number of viable knockout animals have been constructed for various genes so far. Among autoimmune diseases, for example, a thyrotropin receptor (TSH-R) gene knockout mouse (thyrotropin receptor gene being a causative antigen of hyperthyroidism in Grave's disease) has been constructed (see Marians et al., "Defining Thyrotropin-dependent and –Independent Steps of Thyroid Hormone Synthesis by Using Thyrotropin Receptor-Null Mice," Proc Natl Acad Sci USA 99:15776-15781 (2002) (attached hereto as Appendix A)). Thus, one of ordinary skill in the art is readily able to either make or otherwise obtain knockout animals suitable for practicing the present invention.

With regard to item (2), it is the position of the PTO that claim 2 and its dependent claims, which encompass making an autoimmune animal model by transplanting *non-activated* immune cells from a donor to a recipient animal, are not enabled. Applicants respectfully disagree.

As demonstrated in the accompanying Declaration of Masayuki Amagai Under 37 C.F.R. § 1.132 ("Amagai Declaration") as well as the exhibits referenced therein, one of ordinary skill in the art, having read the present application, would have been readily able to make and use the present invention as claimed in claim 2 and its dependent claims.

In particular, the Amagai Declaration demonstrates that researchers could prepare a mammalian model of an autoimmune disease by transferring naïve (i.e., non-activated) immune cells from a donor mammal deficient in a gene encoding an antigen protein of an autoimmune disease to a recipient mammal in accordance with the disclosure of the above-identified patent application and, in fact, have done so (Amagai Declaration ¶ 5). In particular, the Amagai Declaration shows that it is possible to make an autoimmune animal model for pemphigus vulgaris by transferring naïve immune cells from a $Dsg3^{-/-}$ donor mammal to a recipient mammal expressing the Dsg3 protein (Id.).

To determine whether the step of immunizing a $Dsg3^{-/-}$ mouse with recombinant Dsg3 (rDsg3) protein is critical for constructing a model mouse that produces anti-Dsg3 IgG and exhibits the phenotype of pemphigus vulgaris, the transfer of splenocytes

from a naïve $Dsg3^{-/-}$ mouse (i.e., not immunized with Dsg3 protein) into a recipient mouse was conducted (Amagai Declaration ¶ 6). $DSG3^{-/-}$ mice were prepared by mating male $DSG3^{-/-}$ mice with female $DSG3^{+/-}$ mice, and were used as donors (Koch et al., J. Cell Biol. 137:1091-1102 (1997)) (attached to the Amagai Declaration as Exhibit A) (Id.). $RAG2^{-/-}$ mice, which had been obtained by back-crossing with B6.SJL-$ptpr^c$ over 10 generations, were provided by Taconic (German Town, NY) (Schulz et al., J. Immunol. 157:4379-4389 (1996)) (attached to the Amagai Declaration as Exhibit B), and were used as recipients (Id.). Such $RAG2^{-/-}$ mice express Dsg3 protein, but do not reject transplanted splenocytes, because they are deficient in rearrangement of T cell receptor genes and immunoglobulin genes, thereby having neither mature T cells nor B cells (Id.).

Splenocytes were isolated from naïve $Dsg3^{-/-}$ mice by a conventional method. (Amagai Declaration ¶ 7). To perform the adoptive transfer of splenocytes, monocytes were isolated from the spleens of $DSG3^{-/-}$ mice and re-suspended in complete RPMI 1640 medium (Nissui Pharmaceuticals, Tokyo) containing 10% fetal bovine serum, 0.21% sodium bicarbonate solution (w/v), 2 mM L-glutamine (GIBCO), and antibiotics (Id.). About 2×10^7 or about 5×10^7 splenocytes were suspended in PBS and transferred into a $RAG2^{-/-}$ mouse via a caudal vein by intravenous injection (each n=20) (Id.). The production of antibody was tested by ELISA using rDsg3 as a coating antigen (Id.).

To carry out an ELISA assay for blood IgG against mouse Dsg3 protein (mDsg3), a 96-well microtiter plate was coated with 100 µl of 5 µg/ml purified mouse rDsg3 at 4°C overnight (Amagai Declaration ¶ 8). Recipient serum samples were diluted 50 to 5,000 times and then incubated on the 96-well ELISA plate at room temperature for 1 hour (Id.). After the samples were incubated with peroxidase-conjugated anti-mouse IgG antibody (MBL, Nagoya, Japan) at room temperature for 1 hour, the coloring reaction was carried out by using 1 mM tetramethylbenzidine as a substrate for peroxidase (Ishii et al., J Immunol 159:2010-2017 (1997) (attached to the Amagai Declaration as Exhibit C); Amagai et al., Br J Dermatol 140:351-357 (1999) (attached to the Amagai Declaration as Exhibit D)) (Id.). The time course of OD_{450} change in each sample was analyzed in duplicate (Id.). Results in mouse #799, #800, and #317 which were transplanted with 2×10^7 splenocytes per recipient mouse, and #310 and #337 which were transplanted with 5×10^7 splenocytes per recipient mouse are shown in Figure 1 (attached to the Amagai Declaration as Exhibit E) (Id.). Except for #317, anti-Dsg3 IgG was detected in the blood of recipient $RAG2^{-/-}$ mice at day 14 after the transfer of $DSG3^{-/-}$ splenocytes (Id.). The antibody production was rapidly increased and

reached a plateau around day 28 (Id.). Antibody production then continued permanently (Id.). The sustained antibody production was observed for 6 months or more as long as the mice were alive (Id.).

Further, the production of antibody against Dsg3 protein was tested by immuno-fluorescent staining of cultured keratinocytes (Amagai Declaration ¶ 9). Mouse keratinocytes from cell line PAM212 (Yuspa et al., Cancer Res. 40:4694-4703 (1980)) (attached to the Amagai Declaration as Exhibit F) were incubated with recipient mouse serum sample diluted 20-fold with DMEM containing 10% fetal calf serum at 37°C under humid air containing 5% CO_2 for 30 minutes (Id.). Subsequently, the cells were washed with PBS(-), fixed with 100% methanol at -20°C for 20 minutes, and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody (DAKO, Copenhagen, Denmark) at room temperature for 30 minutes (Id.). The stain was observed using a fluorescence microscope (Nikon, Eclipse E800) (Id.). As a result, *in vivo* IgG deposition was found on the cell surface of stratified squamous epithelium keratinocytes in the recipient $RAG2^{-/-}$ mouse (Id.). This result is shown in Figure 2 (attached to the Amagai Declaration as Exhibit G) (Id.).

In recipient mice that were transplanted with 2×10^7 splenocytes per mouse, the production of anti-Dsg3 antibody was detected at 2 weeks after the transfer in 12 out of the 20 mice examined by ELISA, and the phenotype of pemphigus vulgaris was apparent at 4 weeks after the transfer in 11 mice (Amagai Declaration ¶ 10). In recipient mice that were transplanted with 5×10^7 splenocytes per mouse, the titer of the antibody was increased at 2 weeks after the transfer in 16 out of the 20 mice examined, and the phenotype was apparent at 3 weeks after the transfer in 15 mice (Id.). In the mice exhibiting the pemphigus vulgaris phenotype, IgG deposition was found in the inter-cell region of the mucous membrane of the hard palate, as shown in Figure 3A (attached to the Amagai Declaration as Exhibit H) (Id.). Acantholysis immediately above the basal layer, which is a typical characteristic of pemphigus vulgaris, was also observed, as seen in Figure 3B (attached to the Amagai Declaration as Exhibit H) (Id.). The mice showing the phenotype described above also exhibited extensive hair loss, submaxillar and plantar elosion, and scab-formation, which are characteristics of the major phenotype of pemphigus vulgaris (Id.). This is shown in Figure 4 (attached to the Amagai Declaration as Exhibit I) (Id.).

Accordingly, it is apparent that splenocytes of a naïve $Dsg3^{-/-}$ mammal are capable of being activated by contact with the endogenous Dsg3 of a recipient mammal after

the transplantation of non-immunized splenocytes from the $Dsg3^{-/-}$ mammal to the recipient mammal (Amagai Declaration ¶ 11). It is further apparent that the production of anti-Dsg3 antibody and exhibition of the phenotype of pemphigus vulgaris is induced in the recipient mammal (Id.).

As demonstrated by all the foregoing, it is possible to make an autoimmune non-human mammalian model by transplanting immune cells from a naïve antigen gene-deficient donor to a recipient, thereby inducing anti-antigen antibody production in the recipient animal, which results in the creation of an autoimmune disease condition phenotype in the recipient mammal, as claimed in the above-identified application (Amagai Declaration ¶ 12).

The steps of isolating and transferring immune cells from a non-human mammalian donor lacking a gene encoding an antigen protein of an autoimmune disease to a non-human mammalian recipient, and testing for production an antibody reactive to the antigen protein in the recipient are fully described in the present application at page 11, line 16 to page 15, line 35 of the specification.

With regard to items (3) and (4), the PTO states that transplanting immune cells between immune competent non-human mammals and between different species or different individuals within the same species would "trigger an allogenic or xenogenic transplantation response" in carrying out the claimed invention (office action at page 4, first full para.) Therefore, the PTO argues that claims 2, 3 and 11 are not enabled. Applicants submit that claims 2, 3, and 11, as amended, include the limitations "wherein the recipient (i) is the same species as the donor", and (ii) wherein the recipient "has the same genetic background and/or is immunodeficient" which overcomes this basis for rejection.

Moreover, the PTO argues that when transplanting immune cells from a xenogenic source, a phenotype of autoimmune disease may not show and, therefore, the claims are not enabled. Applicants have amended independent claims 2, 3 and 11 to remove the requirement that the non-human mammal "show a phenotype of autoimmune disease." Accordingly, this basis for rejection is overcome.

Thus, for all the foregoing reasons, applicants submit that claims 2-25, as amended, are fully enabled and that the rejection of claims 2-25 under 35 U.S.C. § 112 (1st para.) for lack of enablement is improper and should be withdrawn.

The rejection of claims 2-25 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 2-25 under 35 U.S.C. § 102(a) as anticipated by Amagai et al., J Clin Invest 105:625-31 (2000), is respectfully traversed in view of the translation of the foreign priority application submitted herewith.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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